CALCILYTIC COMPOUNDS

FIELD OF INVENTION

The present invention relates to novel calcilytic compounds, pharmaceutical compositions containing these compounds and their use as calcium receptor antagonists.

In mammals, extracellular Ca^{2+} is under rigid homeostatic control and regulates various processes such as blood clotting, nerve and muscle excitability, and proper bone formation. Extracellular Ca^{2+} inhibits the secretion of parathyroid hormone ("PTH") from parathyroid cells, inhibits bone resorption by osteoclasts, and stimulates secretion of calcitonin from C-cells. Calcium receptor proteins enable certain specialized cells to respond to changes in extracellular Ca^{2+} concentration.

PTH is the principal endocrine factor regulating Ca^{2+} homeostasis in the blood and extracellular fluids. PTH, by acting on bone and kidney cells, increases the level of Ca^{2+} in the blood. This increase in extracellular Ca^{2+} then acts as a negative feedback signal, depressing PTH secretion. The reciprocal relationship between extracellular Ca^{2+} and PTH secretion forms an important mechanism maintaining bodily Ca^{2+} homeostasis.

Extracellular Ca^{2+} acts directly on parathyroid cells to regulate PTH secretion. The existence of a parathyroid cell surface protein which detects changes in extracellular Ca^{2+} has been confirmed. See Brown et al., Nature 366:574, 1993. In parathyroid cells, this protein, the calcium receptor, acts as a receptor for extracellular Ca^{2+}, detects changes in the ion concentration of extracellular Ca^{2+} , and initiates a functional cellular response, PTH secretion.

Extracellular Ca^{2+} influences various cell functions, reviewed in Nemeth et al., Cell Calcium 11:319, 1990. For example, extracellular Ca^{2+} plays a role in parafollicular (C-cells) and parathyroid cells. See Nemeth, Cell Calcium 11:323, 1990. The role of extracellular Ca^{2+} on bone osteoclasts has also been studied. See Zaidi, Bioscience Reports 10:493, 1990.

Various compounds are known to mimic the effects of extra-cellular Ca^{2+} on a calcium receptor molecule. Calcilytics are compounds able to inhibit calcium receptor activity, thereby causing a decrease in one or more calcium receptor activities evoked by extracellular Ca^{2+}. Calcilytics are useful as lead molecules in the discovery, development, design, modification and/or construction of useful calcium modulators, which are active at Ca^{2+} receptors. Such calcilytics are useful in the treatment of various disease states characterized by abnormal levels of one or more components, e.g., polypeptides such as

hormones, enzymes or growth factors, the expression and/or secretion of which is regulated or affected by activity at one or more Ca^{2+} receptors. Target diseases or disorders for calcilytic compounds include diseases involving abnormal bone and mineral homeostasis.

Abnormal calcium homeostasis is characterized by one or more of the following activities: an abnormal increase or decrease in serum calcium; an abnormal increase or decrease in urinary excretion of calcium; an abnormal increase or decrease in bone calcium levels (for example, as assessed by bone mineral density measurements); an abnormal absorption of dietary calcium; an abnormal increase or decrease in the production and/or release of messengers which affect serum calcium levels such as PTH and calcitonin; and an abnormal change in the response elicited by messengers which affect serum calcium levels.

Thus, calcium receptor antagonists offer a unique approach towards the pharmacotherapy of diseases associated with abnormal bone or mineral homeostasis, such as hypoparathyroidism, osteosarcoma, periodontal disease, fracture healing, osteoarthritis, rheumatoid arthritis, Paget's disease, humoral hypercalcemia associated with malignancy and fracture healing, and osteoporosis.

SUMMARY OF THE INVENTION

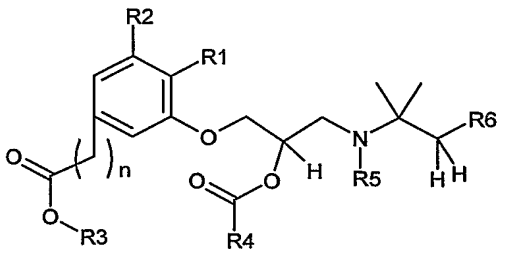
The present invention comprises novel calcium receptor antagonists represented by Formula (I) hereinbelow and their use as calcium receptor antagonists in the treatment of a variety of diseases associated with abnormal bone or mineral homeostasis, including but not limited to hypoparathyroidism, osteosarcoma, periodontal disease, fracture healing, osteoarthritis, rheumatoid arthritis, Paget's disease, humoral hypercalcemia associated with malignancy and fracture healing, and osteoporosis.

The present invention further provides a method for antagonizing calcium receptors in an animal, including humans, which comprises administering to an animal in need thereof an effective amount of a compound of Formula (I), indicated hereinbelow.

The present invention further provides a method for increasing serum parathyroid levels in an animal, including humans, which comprises administering to an animal in need thereof an effective amount of a compound of Formula (I), indicated herein below.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention are selected from Formula (I) herein below:



R1 is selected from the group consisting of H, CN, and halogen;

R2 is selected from the group consisting of halogen and H;

R3 is selected from the group consisting of H and C_{1-5} alkyl, optionally substituted;

n is 0 – 5;

R4 is selected from the group consisting of C_{1-7} alkyl and cycloalkyl;

R5 is H or COR4; and

R6 is selected from the group consisting of aryl, fused aryl, dihydro, tetrahydro fused aryl, and heteroaryl, unsubstituted or substituted, with any substituent selected from the group consisting of OH, halogen, C_{1-4} alkyl, C_{1-4} alkoxy, CF_3, OCF_3, CN and NO_2.

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined by single carbon-carbon bonds and having 1-20 carbon atoms joined together. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Preferably, substituents on optionally substituted alkyl are selected from the group consisting of aryl, CO_2R, CO_2NHR, OH, OR, CO, NH_2, halo, CF_3, OCF_3 and NO_2, wherein R represents H, C_{1-4} alkyl, C_{3-6} cycloalkyl, C_{2-5} alkenyl, C_{2-5} alkynyl, heterocycloalkyl, or aryl. Additional substituents are selected from F, Cl, Br, I, N, S and O. Preferably, no more than three substituents are present. More preferably, the alkyl has 1-12 carbon atoms and is unsubstituted. Preferably, the alkyl group is linear.

As used herein "cycloalkyl" refers to optionally substituted 3-7 membered carbocyclic rings wherein any substituents are selected from the group consisting of, F, Cl, Br, I, $N(R_4)_2$, SR_4 and OR_4, unless otherwise indicated.

As used herein, "aryl" refers to an optionally substituted aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. Aryl includes carbocyclic aryl, and biaryl groups, all of which may be optionally substituted. Preferred aryl include phenyl and naphthyl. More preferred aryl

include phenyl. Preferred substituents are selected from the group consisting of halogen, C_{1-4} alkyl, OCF_3, CF_3, OMe, CN, OSO_2 R and NO_2, wherein R represents C_{1-4} alkyl or C_{3-6} cycloalkyl.

As used herein, "heteroaryl" refers to an aryl ring containing 1,2 or 3 heteroatoms such as N, S, or O.

As used herein, "alkenyl" refers to an optionally substituted hydrocarbon group containing at least one carbon-carbon double bond and containing up to 5 carbon atoms joined together. The alkenyl hydrocarbon chain may be straight, branched or cyclic. Any substituents are selected from the group consisting of halogen, C_{1-4} alkyl, OCF_3, CF_3, OMe, CN, OSO_2 R and NO_2, wherein R represents C_{1-4} alkyl or C_{3-6} cycloalkyl.

As used herein, "alkynyl" refers to an optionally substituted hydrocarbon group containing at least one carbon-carbon triple bond between the carbon atoms and containing up to 5 carbon atoms joined together. The alkynyl hydrocarbon group may be straight-chained, branched or cyclic. Any substituents are selected from the group consisting of halogen, C_{1-4} alkyl, OCF_3, CF_3, OMe, CN, OSO_2 R and NO_2, wherein R represents C_{1-4} alkyl or C_{3-6} cycloalkyl.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

Preferred compounds of the present inventions include:

3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(3-methylbutanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride;

3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2-methylpropanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride;

3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2,2-dimethylpropanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride;

3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4-cyanophenyl}propanoic acid hydrochloride;

3-{4-cyano-3-[((2*R*)-2-[(cyclopropylcarbonyl)oxy]-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]phenyl}propanoic acid hydrochloride;

3-(4-cyano-3-{[(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-(D-valyloxy)propyl]oxy}phenyl)propanoic acid hydrochloride;

3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(3-methylbutanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid trifluoroacetate;

3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2-methylpropanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid trifluoroacetate;

ethyl 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4-cyanophenyl}propanoate hydrochloride;

ethyl 3-(3-{[(2*R*)-3-{acetyl[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-(acetyloxy)propyl]oxy}-4-cyanophenyl)propanoate;

(1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 2-methylpropanoate hydrochloride;

(1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 3-methylbutanoate hydrochloride;

(1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 2,2-dimethylpropanoate hydrochloride;

(1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl cyclopropanecarboxylate hydrochloride;

ethyl 3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(trifluoroacetyl)oxy]propyl}oxy)phenyl]propanoate;

3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2,2-dimethylpropanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid;

3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(phenylcarbonyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid;

3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4,5-difluorophenyl}propanoic acid;

(1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 3-methylbutanoate;

(1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 2-methylpropanoate;

(1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 2,2-dimethylpropanoate;

(1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl benzoate;

ethyl 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4,5-difluorophenyl}propanoate.

Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered.

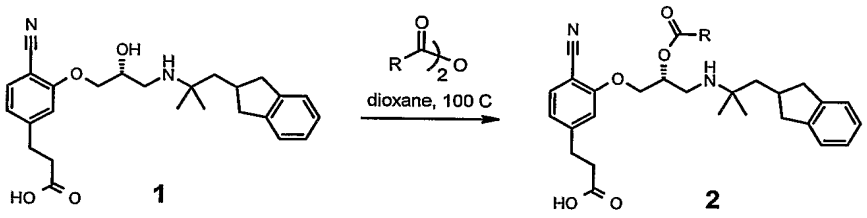
Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. A preferred salt is a hydrochloride. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present.

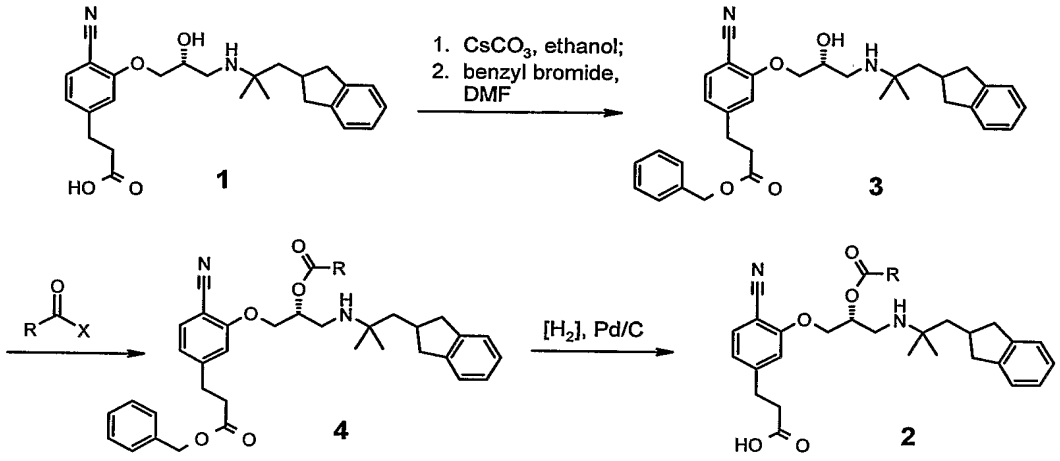
The present invention provides compounds of Formula (I) above, which can be prepared using standard techniques. An overall strategy for preparing preferred compounds described herein can be carried out as described in this section. The examples, which follow, illustrate the synthesis of specific compounds. Using the protocols described herein as a model, one of ordinary skill in the art can readily produce other compounds of the present invention.

All reagents and solvents were obtained from commercial vendors. Starting materials were synthesized using standard techniques and procedures.

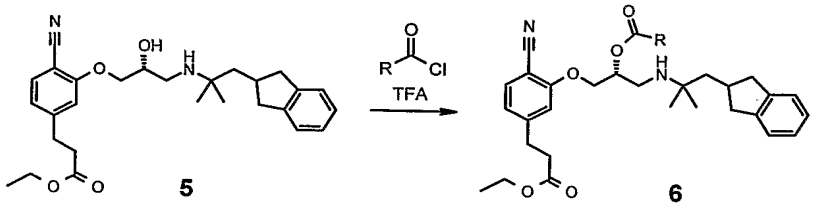
Scheme 1



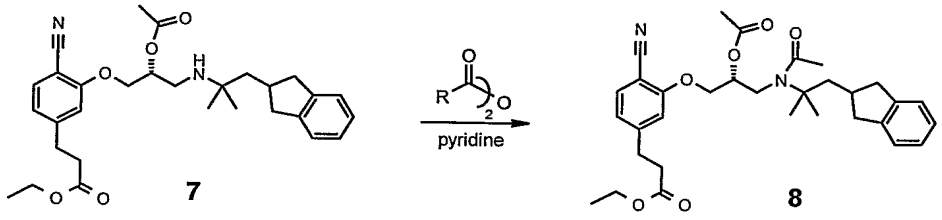
Scheme 2 Alternate procedures for preparation of mono-esters:



Scheme 3 Preparation of bis esters:



Scheme 4 Preparation of amide - ester:



General Preparation:

The synthesis of the compounds of the general formula (I) may be prepared as outlined above in Schemes 1-4.

Treatment of the carboxylic acid **1** with an acid anhydride such as acetic anhydride in dioxane under neutral conditions provides the acid – ester **2** after aqueous work up as shown in Scheme 1. Alternatively, as depicted in Scheme 2, the carboxylic acid of compound **1** can first be protected by treatment with a base such as Cs_2CO_3 and an alkylating agent such as benzyl bromide to give intermediate ester **3**. The alcohol **3** can then be converted to an ester **4** using conditions common to the art such as treatment with an activated carboxylic acid such as an acid chloride, an acid anhydride, or an acid in the presence of a carbodiimide such as EDC. The benzyl ester **4** is deprotected under conditions which are common to the art such as hydrogen or a hydrogen transfer agent such as cyclohexene in the presence of a catalyst such as palladium on carbon to provide the acid **2**.

The double esters **6** can be provided in one step from ester **5** by treatment with an acid chloride in a solvent such as trifluoroacetic acid (Scheme 3). The double ester **7** can be further modified using conditions common to the art such as treatment with an acid anhydride in pyridine to provide the amide **8**, as shown in Scheme 4.

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The calcilytic compounds can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, topical (transdermal), or transmucosal administration. For systemic administration, oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets, and liquid preparations such as syrups, elixirs, and concentrated drops.

Alternatively, injection (parenteral administration) may be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably, in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and

redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art.

The amounts of various calcilytic compounds to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50}, EC_{50}, the biological half-life of the compound, the age, size and weight of the patient, and the disease or disorder associated with the patient. The importance of these and other factors to be considered are known to those of ordinary skill in the art.

Amounts administered also depend on the routes of administration and the degree of oral bioavailability. For example, for compounds with low oral bioavailability, relatively higher doses will have to be administered.

Preferably, the composition is in unit dosage form. For oral application, for example, a tablet, or capsule may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. In each case, dosing is such that the patient may administer a single dose.

Each dosage unit for oral administration contains suitably from 0.01 to 500 mg/Kg, and preferably from 0.1 to 50 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. The daily dosage for parenteral, nasal, oral inhalation, transmucosal or transdermal routes contains suitably from 0.01 mg to 100 mg/Kg, of a compound of Formula (I). A topical formulation contains suitably 0.01 to 5.0% of a compound of Formula (I). The active ingredient may be administered, for example, from 1 to 6 times per day, preferably once, sufficient to exhibit the desired activity, as is readily apparent to one skilled in the art.

As used herein, "treatment" of a disease includes, but is not limited to prevention, retardation and prophylaxis of the disease.

Diseases and disorders which might be treated or prevented, based upon the affected cells, include bone and mineral-related diseases or disorders; hypoparathyroidism; those of the central nervous system such as seizures, stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, such as occurs in cardiac arrest or neonatal distress, epilepsy, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease, dementia, muscle tension, depression, anxiety, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, schizophrenia, neuroleptic malignant syndrome, and Tourette's syndrome; diseases involving excess water reabsorption by the kidney, such as syndrome of inappropriate ADH secretion (SIADH), cirrhosis, congestive heart failure, and nephrosis; hypertension; preventing and/or decreasing renal toxicity from cationic antibiotics (e.g., aminoglycoside antibiotics); gut motility disorders such as diarrhea and spastic colon; GI ulcer diseases; GI diseases with excessive calcium absorption such as sarcoidosis; autoimmune diseases and organ transplant rejection; squamous cell carcinoma; and pancreatitis.

In a preferred embodiment of the present invention, the present compounds are used to increase serum parathyroid hormone ("PTH") levels. Increasing serum PTH levels can be helpful in treating diseases such as hypoparathyroidism, osteosarcoma, periodontal disease, fracture, osteoarthritis, rheumatoid arthritis, Paget's disease, humoral hypercalcemia malignancy and osteoporosis.

In a preferred embodiment of the present invention, the present compounds are co-administered with an anti-resorptive agent. Such agents include, but are not limited estrogen, 1, 25 $(OH)_2$ vitamin D3, calcitonin, selective estrogen receptor modulators, vitronectin receptor antagonists, V-H+-ATPase inhibitors, src SH2 antagonists, bisphosphonates and cathepsin K inhibitors.

Another aspect of the present invention describes a method of treating a patient comprising administering to the patient an amount of a present compound sufficient to increase the serum PTH level. Preferably, the method is carried out by administering an amount of the compound effective to cause an increase in duration and/or quantity of serum PTH level sufficient to have a therapeutic effect.

In various embodiments, the compound administered to a patient causes an increase in serum PTH having a duration of up to one hour, about one to about twenty-four hours,

about one to about twelve hours, about one to about six hours, about one to about five hours, about one to about four hours, about two to about five hours, about two to about four hours, or about three to about six hours.

In an alternative embodiment of the present invention, the compound administered to a patient causes an increase in serum PTH having a duration of more than about twenty four hours provided that it is co-administered with an anti resorptive agent.

In additional different embodiments, the compound administered to a patient causes an increase in serum PTH of up to two fold, two to five fold, five to ten fold, and at least 10 fold, greater than peak serum PTH in the patient. The peak serum level is measured with respect to a patient not undergoing treatment.

Composition of Formula (I) and their pharmaceutically acceptable salts, which are active when given orally, can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way,

with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

No unacceptable toxological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the following tests:

(I) Calcium Receptor Inhibitor Assay

Calcilytic activity was measured by determining the IC_{50} of the test compound for blocking increases of intracellular Ca^{2+} elicited by extracellular Ca^{2+} in HEK 293 4.0-7 cells stably expressing the human calcium receptor. HEK 293 4.0-7 cells were constructed as described by Rogers et al., J. Bone Miner. Res. 10 Suppl. 1:S483, 1995 (hereby incorporated by reference herein). Intracellular Ca^{2+} increases were elicited by increasing extracellular Ca^{2+} from 1 to 1.75 mM. Intracellular Ca^{2+} was measured using fluo-3, a fluorescent calcium indicator.

The procedure was as follows:

1. Cells were maintained in T-150 flasks in selection media (DMEM supplemented with 10% fetal bovine serum and 200 ug/mL hygromycin B), under 5% CO_2:95% air at 37 ^oC and were grown up to 90% confluency.

2. The medium was decanted and the cell monolayer was washed twice with phosphate-buffered saline (PBS) kept at 37 ^oC. After the second wash, 6 mL of 0.02% EDTA in PBS was added and incubated for 4 minutes at 37 ^oC. Following the incubation, cells were dispersed by gentle agitation.

3. Cells from 2 or 3 flasks were pooled and pelleted (100 x g). The cellular pellet was resuspended in 10-15 mL of SPF-PCB+ and pelleted again by centrifugation. This washing was done twice.

Sulfate- and phosphate-free parathyroid cell buffer (SPF-PCB) contains 20 mM Na-Hepes, pH 7.4, 126 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$. SPF-PCB was made up and stored at 4 ^oC. On the day of use, SPF-PCB was supplemented with 1 mg/mL of

D-glucose and 1 mM $CaCl_2$ and then split into two fractions. To one fraction, bovine serum albumin (BSA; fraction V, ICN) was added at 5 mg/mL (SPF-PCB+). This buffer was used for washing, loading and maintaining the cells. The BSA-free fraction was used for diluting the cells in the cuvette for measurements of fluorescence.

4. The pellet was resuspended in 10 mL of SPF-PCB+ containing 2.2 uM fluo-3 (Molecular Probes) and incubated at room temperature for 35 minutes.

5. Following the incubation period, the cells were pelleted by centrifugation. The resulting pellet was washed with SPF-PCB+. After this washing, cells were resuspended in SPF-PCB+ at a density of 1-2 x 106 cells/mL.

6. For recording fluorescent signals, 300 uL of cell suspension were diluted in 1.2 mL of SPF buffer containing 1 mM $CaCl_2$ and 1 mg/mL of D-glucose. Measurements of fluorescence were performed at 37 ^oC with constant stirring using a spectrofluorimeter. Excitation and emission wavelengths were measured at 485 and 535 nm, respectively. To calibrate fluorescence signals, digitonin (5 mg/mL in ethanol) was added to obtain Fmax, and the apparent Fmin was determined by adding Tris-EGTA (2.5 M Tris-Base, 0.3 M EGTA). The concentration of intracellular calcium was calculated using the following equation:

Intracellular calcium = $(F\text{-}F_{min}/F_{max}) \times K_d$; where K_d = 400 nM.

7. To determine the potential calcilytic activity of test compounds, cells were incubated with test compound (or vehicle as a control) for 90 seconds before increasing the concentration of extracellular Ca^{2+} from 1 to 2mM. Calcilytic compounds were detected by their ability to block, in a concentration-dependent manner, increases in the concentration of intracellular Ca^{2+} elicited by extracellular Ca^{2+}.

In general, those compounds having lower IC_{50} values in the Calcium Receptor Inhibitor Assay are more preferred compounds. Compounds having an IC_{50} greater than 50 uM were considered to be inactive. Preferred compounds are those having an IC_{50} of 10uM or lower, more preferred compounds have an IC_{50} of 1uM, and most preferred compounds have an IC_{50} of 0.1uM or lower.

(II) Calcium Receptor Binding Assay

HEK 293 4.0-7 cells stably transfected with the Human Parathyroid Calcium Receptor ("HuPCaR") were scaled up in T180 tissue culture flasks. Plasma membrane is obtained by polytron homogenization or glass douncing in buffer (50mM Tris-HCl pH 7.4,

1mM EDTA, 3mM $MgCl_2$) in the presence of a protease inhibitor cocktail containing 1uM Leupeptin, 0.04 uM Pepstatin, and 1 mM PMSF. Aliquoted membrane was snap frozen and stored at –80 °C. ^3H labeled compound was radiolabeled to a radiospecific activity of 44Ci/mmole and was aliquoted and stored in liquid nitrogen for radiochemical stability.

A typical reaction mixture contains 2 nM ^3H compound ((R,R)-N-4'-Methoxy-t-3-3'-methyl-1'-ethylphenyl-1-(1-naphthyl)ethylamine), or ^3H compound (R)-N-[2-Hydroxy-3-(3-chloro-2-cyanophenoxy)propyl]-1,1-dimethyl-2-(4-methoxyphenyl)ethylamine 4-10 ug membrane in homogenization buffer containing 0.1% gelatin and 10% EtOH in a reaction volume of 0.5 mL. Incubation is performed in 12 x 75 polyethylene tubes in an ice water bath. To each tube 25 uL of test sample in 100% EtOH is added, followed by 400 uL of cold incubation buffer, and 25 uL of 40 nM ^3H-compound in 100% EtOH for a final concentration of 2nM. The binding reaction is initiated by the addition of 50 uL of 80-200 ug/mL HEK 293 4.0-7 membrane diluted in incubation buffer, and allowed to incubate at 4°C for 30 min. Wash buffer is 50 mM Tris-HCl containing 0.1% PEI. Nonspecific binding is determined by the addition of 100-fold excess of unlabeled homologous ligand, and is generally 20% of total binding. The binding reaction is terminated by rapid filtration onto 1% PEI pretreated GF/C filters using a Brandel Harvestor. Filters are placed in scintillation fluid and radioactivity assessed by liquid scintillation counting.

Example 1

Preparation of 3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(3-methylbutanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride

To a suspension of carboxylic acid **1** (prepared according to the literature: WO 2001053254) (2 g, 4.59 mmol, zwitterion) in dioxane (90 mL) was added isovaleric anhydride (1.8 mL, 9.17 mmol). The resulting mixture was heated to 100 °C for 30 minutes, or once complete dissolution was achieved. After 1 h at room temperature, 5% HCl (45 mL) was added, and the mixture stirred for 30 min to hydrolyze the propionate – isovalerate anhydride by-product. The dioxane was removed by rotoevaporation, and the resulting aqueous layer was partitioned with ethyl acetate. The layers were separated and the residual aqueous layer was adjusted to pH 5. The aqueous layer was then extracted 2

times with ethyl acetate. The organic portions were pooled, dried over $MgSO_4$, filtered and concentrated to give 3 g of an orange oil containing approximately 80% of the desired product. Preparative HPLC separation (50% CH_3CN/NH_4OAc buffer, pH5, for 5 minutes; ramp to 90% CH_3CN over 15 minutes; phenomenex column, 250 x 21 mm, 10micron) provided 500 mg pure product (20%) as the HCl salt after treatment with 2M HCl in diethyl ether.

^1H NMR (500 MHz) dmso-d_6: δ 12.16 (br s, 1H); 9.10 (br m, 1H); 8.48 (br m, 1H); 7.62 (d, J = 7.8 Hz, 1H); 7.20 (s, 1H); 7.16 (m, 2H); 7.08 (m, 2H); 6.99 (d, J = 8.3 Hz, 1H); 5.35 (m, 1H); 4.43 (dd, J = 11.2, 3.4 Hz, 1H); 4.35 (dd, J = 11.2, 3.4 Hz, 1H); 3.42 (m, 1H); 3.34 (m, 1H); 3.08 (dd, J = 14.6, 7.3 Hz, 2H); 2.85 (t, J = 7.3 Hz, 2H); 2.59-2.51 (m, 5H); 2.21 (d, J = 7.32 Hz, 2H); 1.96 (m, 3H); 1.39 (s, 3H); 1.38 (s, 3H).

LC/MS: 521 (M+H)

Example 2

Preparation of 3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2-methylpropanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride

To a suspension of carboxylic acid **1** (0.5 g, 1.06 mmol, zwitterion) in dioxane (18 mL, 0.06 M) was added isobutyric anhydride (0.39 mL, 2.32 mmol). The resulting mixture was heated to 60 °C overnight. The dioxane was removed by rotoevaporation, and the resulting residue was purified by reversed-phase HPLC (5% to 95% CH_3CN/H_2O, 10 min gradient; YMC column, 75 x 30 mm, 10 micron). The purified material was treated with 1M HCl in diethyl ether and acetonitrile and dried to provide 0.13 g of the HCl salt (24%).

LC/MS: 507 (M+H)

Example 3

Preparation of 3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2,2-dimethylpropanoyl)oxy]propyl}oxy)phenyl]propanoic acid hydrochloride

To a suspension of carboxylic acid **1** (0.51 g, 1.17 mmol, zwitterion) in dioxane (18 mL, 0.06 M) was added 2,2,2-trimethylacetic anhydride (0.52 mL, 2.57 mmol). The resulting mixture was heated to 40 °C for 3 days. The dioxane was removed by rotoevaporation, and the resulting residue was purified by reversed-phase HPLC (5% to 95% CH_3CN/H_2O, 10 min gradient; YMC column, 75 x 30 mm, 10 micron). The purified material was treated with 1M HCl in diethyl ether and acetonitrile and dried to provide 0.15 g of the HCl salt (23%).
LC/MS: 521 (M+H)

Example 4

Preparation of 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4-cyanophenyl}propanoic acid hydrochloride

To a suspension of carboxylic acid **1** (0.80 g, 1.69 mmol, zwitterion) in dioxane (30 mL) was added acetic anhydride (0.52 mL, 2.57 mmol) and several drops of water. The resulting mixture was heated to 40 °C overnight. The dioxane was removed by rotoevaporation, and the resulting residue was brought up in water and extracted with CH_2Cl_2. The organic portion was dried ($MgSO_4$), filtered, and concentrated to give 0.8 g of crude product. Purification was achieved by reversed-phase HPLC (5% to 95% CH_3CN/H_2O, 10 min gradient; YMC column, 75 x 30 mm, 10 micron). The purified material was treated with 1M HCl in diethyl ether and acetonitrile and dried to provide 0.50 g of the HCl salt containing 20% of starting material as an impurity.
LC/MS: 479 (M+H)

Example 5

Preparation of 3-{4-cyano-3-[((2*R*)-2-[(cyclopropylcarbonyl)oxy]-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]phenyl}propanoic acid hydrochloride

a) A suspension of carboxylic acid **1** (0.83 g, 1.75 mmol) in absoute ethanol (30 mL) was treated with Cs_2CO_3 (1.3 g, 3.87 mmol) in 5 mL water to bring the pH to 8. The resulting suspension was concentrated to dryness in vacuo and azeotroped several times with toluene. The resulting solid was suspended in dry DMF (30 mL) and treated with benzyl bromide (0.33 g, 1.93 mmol). The reaction mixture was heated to 60 °C overnight.

The solvent was reduced by rotoevaporation, and the resulting mixture was partitioned between water and CH_2Cl_2. The organic portion was dried ($MgSO_4$), filtered, and concentrated. FCC purification (SiO_2; 5% MeOH/CH_2Cl_2) provided 0.74 g of material (80%). LCMS (M+H) = 527.

b) A TFA (5 mL) solution of the benzyl ester from Example 5a (0.74 g, 1.4 mmol) was treated with cyclopropylcarbonyl chloride (0.3 mL, 3.1 mmol) and stirred at room temperature overnight. The reaction mixture was concentrated under vacuum, and the product was converted to the free base by treatment with dry K_2CO_3 in CH_2Cl_2 (0.8 g, 96%).

c) A THF (16 mL) solution of the ester from Example 5b (0.4 g, 0.67 mmol) was treated with cyclohexene (8 mL) and 10% palladium on carbon (0.09 g) and heated to reflux overnight. The reaction mixture was then filtered, concentrated under vacuum, and purified by FCC (SiO_2; 10% MeOH/CH_2Cl_2) to provide 0.07 g of pure carboxylic acid (21%).

LC/MS: 505 (M+H)

Example 6

Preparation of 3-(4-cyano-3-{[(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-(D-valyloxy)propyl]oxy}phenyl)propanoic acid hydrochloride

a) A suspension of carboxylic acid **1** (5.0 g, 11.4 mmol) in absolute ethanol (150 mL) was treated with Cs_2CO_3 (6.5 g,) in 25 mL water to bring the pH to 8. The resulting suspension was concentrated to dryness in vacuo and azeotroped several times with toluene. The resulting solid was suspended in dry DMF (150 mL) and treated with benzyl bromide (2.2 g, 12.5 mmol). The reaction mixture was heated to 60 °C overnight. The solvent was reduced by rotoevaporation, and the resulting mixture was partitioned between water and CH_2Cl_2. The organic portion was dried ($MgSO_4$), filtered, and concentrated. FCC purification (SiO2; 5% MeOH/CH2Cl2) provided 5.4 g of material (90%). LCMS (M+H) = 527.

b) To a solution of the benzyl ester Example 6a (1.0 g, 1.9 mmol) in 25 ml CH_2Cl_2 was added Z-L-Valine (0.954g, 3.8 mmol) and EDC (0.730g, 3.8 mmol) at 0 °C. The resulting solution stirred at rt overnight and then washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic portion was dried over $MgSO_4$, filtered, and concentrated. FCC

purification (SiO_2; 90% EtOAc/ Hexene) provided 1.2 g product in 86% yield. LC/MS: 760 (M+H).

c) To a solution of valinate ester from Example 6b (1.2g, 1.58mmol) in 40 mL of ethyl acetate was added 0.6g of 10% Pd/C and 0.5 mL of HCl in diethyl ether (2.0 M) under Ar. The resulting solution was shaken under H_2 (40 psi) at room temperature overnight. The reaction was filtered through Celite and concentrated to give product as white solid (0.84g). The HCl salt was formed by stirring with a solution of HCl in diethyl ether (2.0 M). LC/MS: 536 (M+H)

Example 7

Preparation of 3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(3-methylbutanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid trifluoroacetate

a) A suspension of 3-{3-[((2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-hydroxypropyl)oxy]-4,5-difluorophenyl}propanoic acid hydrochloride (prepared in WO2004047751) (1.0 g, 2.06 mmol) in absolute ethanol (28 mL) was treated with Cs_2CO_3 (1.34 g, 4.12 mmol) in 7 mL water to bring the pH to 8. The resulting suspension was concentrated to dryness in vacuo and azeotroped several times with toluene. The resulting solid was suspended in dry DMF (30 mL) and treated with benzyl bromide (0.27 mL, 2.27 mmol). The reaction mixture was heated to 60 °C overnight. The solvent was reduced by rotoevaporation, and the resulting mixture was partitioned between water and CH_2Cl_2. The organic portion was dried (Na_2SO_4), filtered, and concentrated. FCC purification (SiO_2; 10% MeOH/CH_2Cl_2) provided 0.74 g of material (90%). LCMS (M+H) = 538.

b) A dichloromethane (30 mL) solution of the benzyl ester from Example 7a (1.0 g g, 1.86 mmol) was cooled to 0 °C, treated with isovaleric anhydride (0.37 mL, 1.86 mmol), triethyl amine (0.52 mL, 3.72 mmol), and stirred overnight. The reaction mixture was concentrated under vacuum. FCC purification (SiO_2; 50% EtOAc/Hexanes) produced the diester (0.8 g) in 69% yield. LCMS (M+H) = 623.

c) A THF (32 mL) solution of the benzyl ester (0.8 g, 1.28 mmol) from Example 7b was treated with cyclohexene (16 mL) and 10% palladium on carbon (0.18 g) and heated to reflux overnight. The reaction mixture was then filtered, concentrated under vacuum, and purified by reverse phase HPLC to produce the monoester (0.5 g) in 73% yield. HPLC

purification conditions: YMC column; 75x30 mm ID; 20-90% gradient, CH_3CN/H_2O with 0.1% TFA; 15 min run; desired peak at 9.5 min.
LCMS (M+H) = 532.

Example 8

Preparation of 3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2-methylpropanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid trifluoroacetate

a) A suspension of 3-{3,4-Difluoro-5-[(R)-2-hydroxy-3-(2-indan-2-yl-1,1-dimethyl-ethylamino)-propoxy]-phenyl}-propionic acid (1.0 g, 2.06 mmol) in absolute ethanol (28 mL) was treated with Cs_2CO_3 (1.34 g, 4.12 mmol) in 7 mL water to bring the pH to 8. The resulting suspension was concentrated to dryness in vacuo and azeotroped several times with toluene. The resulting solid was suspended in dry DMF (30 mL) and treated with benzyl bromide (0.27 mL, 2.27 mmol). The reaction mixture was heated to 60 °C overnight. The solvent was reduced by rotoevaporation, and the resulting mixture was partitioned between water and CH_2Cl_2. The organic portion was dried (Na_2SO_4), filtered, and concentrated. FCC purification (SiO_2; 10% MeOH/CH_2Cl_2) provided 0.74 g of material (90%). LCMS (M+H) = 538.

b) A CH_2Cl_2 (30 mL) solution of the benzyl ester from Example 8a (1.0 g g, 1.86 mmol) was cooled to 0 °C treated with isobutyric anhydride (0.31 mL, 1.86 mmol), triethyl amine (0.52 mL, 3.72 mmol) and stirred overnight. The reaction mixture was concentrated under vacuum. FCC purification (SiO_2; 50% EtOAc/Hexanes) produced the diester (0.89 g) in 69% yield. LCMS (M+H) = 608.

c) A THF (32 mL) solution of the benzyl ester (0.89 g, 1.46 mmol) from tExample 8b was treated with cyclohexene (16 mL) and 10% palladium on carbon (0.18 g) and heated to reflux overnight. The reaction mixture was then filtered, concentrated under vacuum, and purified by reverse phase HPLC to produce monoester (0.35 g) in 46% yield. HPLC purification conditions: YMC column; 75x30 mm ID; 20-90 gradient, CH_3CN/H_2O with 0.1% TFA; 15 min run; desired peak at 9.4 min.
LCMS (M+H) = 519.

Example 9

Preparation of Ethyl 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4-cyanophenyl}propanoate hydrochloride

To a solution of ester **5** (prepared by Fischer esterification of carboxylic acid **1**) (0.50 g, 1.0 mmol) in neat trifluoroacetic acid (2 mL) was added acetyl chloride (0.16 mL, 2.20 mmol). This solution stirred at room temperature for 2 hours, and then the solvent was removed by rotoevaporation to give the product as a TFA salt. The residue was brought up in dichloromethane, stirred with solid K_2CO_3, filtered and concentrated to give the free amine. The HCl salt was prepared by dissolving the amine in acetonitrile and adding 2N HCl in diethyl ether. Removal of the solvents gave the bis ester HCl salt as a yellow oil (0.54 g, quant).

LC/MS: 507 (M+H)

Example 10

Preparation of Ethyl 3-(3-{[(2*R*)-3-{acetyl[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-(acetyloxy)propyl]oxy}-4-cyanophenyl)propanoate

To a solution of mono-acetylated ester from Example 9 (0.30 g, 0.59 mmol) in CH_2Cl_2 (5.8 mL) was added DMAP (0.087 g, 0.71 mmol), TEA (0.10 mL, 0.72 mmol) and acetyl chloride (0.052 mL, 0.73 mmol). The reaction mixture was stirred under argon at ambient temperature for 3.5 h. The reaction mixture was then concentrated in vacuo. Column chromatography (3% CH_3OH/CH_2Cl_2) afforded 0.26 g (78%) of the title amide as a white solid.

LC/MS: 549.2 $(M + H)^+$.

Example 11

Preparation of (1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 2-methylpropanoate hydrochloride

To a solution of ester **5** (1.0 g, 2.0 mmol) in neat trifluoroacetic acid (4 mL) was added isobutyl chloride (0.46 mL, 4.4 mmol). This solution stirred at room temperature overnight, and then the solvent was removed by rotoevaporation to give the product as a TFA salt. The residue was brought up in dichloromethane, stirred with solid K_2CO_3, filtered and concentrated to give the free amine. The HCl salt was prepared by dissolving the amine in acetonitrile and adding 2N HCl in diethyl ether. Removal of the solvents gave the bis ester HCl salt as a yellow oil.

LC/MS: 535 (M+H)

Example 12

Preparation of (1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 3-methylbutanoate hydrochloride

To a solution of ester **5** (1.0 g, 2.0 mmol) in neat trifluoroacetic acid (4 mL) was added isovaleryl chloride (0.54 mL, 4.4 mmol). This solution stirred at room temperature overnight, and then the solvent was removed by rotoevaporation to give the product as a TFA salt. The residue was brought up in dichloromethane, stirred with solid K_2CO_3, filtered and concentrated to give the free amine. The HCl salt was prepared by dissolving the amine in acetonitrile and adding 2N HCl in diethyl ether. Removal of the solvents gave the bis ester HCl salt as a yellow oil.

LC/MS: 549 (M+H)

Example 13

Preparation of (1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl 2,2-dimethylpropanoate hydrochloride

To a solution of ester **5** (1.0 g, 2.0 mmol) in neat trifluoroacetic acid (4 mL) was added 2,2,2-trimethylacetyl chloride (0.46 mL, 4.4 mmol). This solution stirred at room temperature overnight, and then the solvent was removed by rotoevaporation to give the product as a TFA salt. The residue was brought up in dichloromethane, stirred with solid

K_2CO_3, filtered and concentrated to give the free amine. The HCl salt was prepared by dissolving the amine in acetonitrile and adding 2N HCl in diethyl ether. Removal of the solvents gave the bis ester HCl salt as a yellow oil. LC/MS: 549 (M+H).

Example 14

Preparation of (1*R*)-2-({2-cyano-5-[3-(ethyloxy)-3-oxopropyl]phenyl}oxy)-1-({[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}methyl)ethyl cyclopropanecarboxylate hydrochloride

To a solution of ester **5** (1.0 g, 2.0 mmol) in neat trifluoroacetic acid (4 mL) was added cyclopropylcarbonyl chloride (0.40 mL, 4.4 mmol). This solution stirred at room temperature overnight, and then the solvent was removed by rotoevaporation to give the product as a TFA salt. The residue was brought up in dichloromethane, stirred with solid K_2CO_3, filtered and concentrated to give the free amine. The HCl salt was prepared by dissolving the amine in acetonitrile and adding 2N HCl in diethyl ether. Removal of the solvents gave the bis ester HCl salt as a yellow oil.
LC/MS: 533 (M+H)

Example 15

Preparation of Ethyl 3-[4-cyano-3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(trifluoroacetyl)oxy]propyl}oxy)phenyl]propanoate

To a solution of ester **5** (0.049 g, 0.098 mmol) in CH_2Cl_2 (1.1 mL) was added trifluoroacetic anhydride (0.09 mL, 0.64 mmol) and pyridine (0.08 mL, 0.99 mmol). The reaction turned bright yellow and was stirred at ambient temperature for 2.5 h. The reaction was diluted with CH_2Cl_2 and washed successively with water, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (1% CH_3OH/CH_2Cl_2) yielded 0.038 g (69%) of the title ester as a yellow solid.

Example 16

Preparation of 3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(2,2-dimethylpropanoyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid trifluoroacetate

This analog was prepared following the general procedure of Example 8a-c except substituting trimethylacetic anhydride for isobutyric anhydride. Purification was accomplished by recrystalization from acetonitrile. The resulting free base was taken up in dry acetonitrile and treated with trifluoroacetic acid to provide the salt in 69% yield. MS(ES) m/e 531, 532.4 $[M+H]^+$.

Example 17

Preparation of 3-[3-({(2*R*)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-2-[(phenylcarbonyl)oxy]propyl}oxy)-4,5-difluorophenyl]propanoic acid hydrochloride

This analog was prepared following the general procedure of Example 8a-c except substituting benzoic anhydride for isobutyric anhydride. Purification was accomplished by recrystalization from ACN/THF (1:1) mixture. The resulting free base was taken up in dry ACN and treated with HCl in 1,4-dioxane to provide the hydrochloride salt in 46% yield. MS(ES) m/e 551, 552.4 $[M+H]^+$.

Example 18

Preparation of 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4,5-difluorophenyl}propanoic acid

This analog was prepared following the general procedure of Example 8a-c except substitutingacetic anhydride for isobutyric anhydride. The crude mixture was purified by HPLC (eluted with ACN/H_2O containing 0.1% TFA) to produce the desired product (0.163 g) in 47% yield. MS(ES) m/e 489, 490.4 $[M+H]^+$.

Example 19

Preparation of (1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 3-methylbutanoate hydrochloride

The HCl salt of the ethyl ester (**5**, 1.0 g, 1.95 mmoles) was dissolved in dichloromethane (19 mL) and cooled to 0°C. To this was added isovaleric anhydride (0.39 mL, 1.95 mmoles) and triethyl amine (0.27 mL, 3.90 mmoles) sequentially. The reaction stirred overnight while warmed to ambient temperature. The reaction was concentrated and purified by flash column chromatography (50% EtOAc/Hexanes) to yield the desired product (0.60 g) in 56% yield. The acylated ethyl ester is taken up in dry ethyl ether and was treated with HCl (1.5 mL; 1M dissolved in ether). The reaction mixture was stirred for 15 minutes and concentrated to provide the hydrochloride salt. MS(ES) m/e 559, 560.4 $[M+H]^+$.

Example 20

Preparation of (1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 2-methylpropanoate

This analog was prepared following the general procedure of Example 19 using ethyl ester (**5**, 1.0 g, 1.95 mmoles) and isobutyric anhydride (0.32 mL, 1.95 mmoles) in 75% (0.80 g) yield. MS(ES) m/e 545, 546.4 $[M+H]^+$.

Example 21

Preparation of (1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl 2,2-dimethylpropanoate

This analog was prepared following the general procedure of Example 19 from the ethyl ester (**5**, 1.0 g, 1.95 mmoles) and trimethylacetic anhydride (0.39 mL, 1.95 mmoles) in 75% (0.82 g) yield. MS(ES) m/e 559, 560.4 $[M+H]^+$.

Example 22

Preparation of (1*R*)-2-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}-1-[({5-[3-(ethyloxy)-3-oxopropyl]-2,3-difluorophenyl}oxy)methyl]ethyl benzoate

This analog was prepared following the general procedure of Example 19 from the ethyl ester (**5**, 0.5 g, 0.98 mmoles) and benzoic anhydride (0.22 g, 0.98 mmoles) in 75% (0.82 g) yield. MS(ES) m/e 579, 580.4 $[M+H]^+$.

Example 23

Preparation of ethyl 3-{3-[((2*R*)-2-(acetyloxy)-3-{[2-(2,3-dihydro-1*H*-inden-2-yl)-1,1-dimethylethyl]amino}propyl)oxy]-4,5-difluorophenyl}propanoate

This analog was prepared following the general procedure of Example 19 from the ethyl ester (**5**, 0.51 g, 1.0 mmoles) and acetic anhydride (0.20 mL, 1.1 mmoles) in 81% (0.42 g) yield. MS(ES) m/e 517, 518.4 $[M+H]^+$.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the area can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.